West Nile Virus Equine Investigation Protocol

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Guidelines for Investigating Suspect West Nile Virus Cases in Equine

USDA-APHIS Veterinary Services

Introduction

In the United States, West Nile virus (WNV) has caused disease and deaths in humans, wild birds, zoo birds, and horses. Wild birds are the reservoir for the virus, which is transmitted by mosquitoes. Limiting exposure to mosquitoes and controlling mosquitoes are fundamental in preventing the disease. The purpose of this document is to guide veterinary practitioners and field personnel in investigating and reporting suspect cases of WNV infection in equine.

Equine Precautions

APHIS Veterinary Services (VS) is concerned about horses and other equine because 25 cases of illness in horses on Long Island, New York, were found to be attributable to WNV in 1999. Nine of those horses died or had to be euthanatized. An additional 36 horses on Long Island were found to have been exposed to WNV and developed antibodies to the virus, but did not develop clinical illness.

To prevent exposure of equine to WNV, it is necessary to prevent their exposure to mosquitoes. No vaccine for WNV is currently available. The most important action to prevent exposure to mosquitoes is source reduction, i.e., the elimination of stagnant water sources where mosquitoes may breed. Insect-proofing stables and other measures that reduce exposure of equine to mosquitoes may be useful in areas where current WNV activity has been documented in mosquitoes, birds, humans, or equine.

Human Precautions

When working with an equine or other mammal showing signs of a central nervous system disorder, always take precautions to avoid exposure to rabies virus. In addition, persons visiting a premises to investigate an unknown disease condition should take measures to prevent exposure to a variety of arthropod-borne zoonotic pathogens. Application of commercially available insect repellants containing DEET to clothing and to exposed parts of the body should be sufficient to protect oneself from mosquitoes carrying WNV.

Equine Surveillance

What should be considered a suspect case of equine WNV infection and how it should be investigated depend on whether or not it occurs in a WNV-affected area.

A WNV-affected area is any county where a WNV infection in an equine has been confirmed in the current calendar year (2000), or any location within 10 miles of a confirmed equine WNV infection. Illness in an equine in a WNV-affected area should be considered a suspect case if at least one of the following signs is present:

- ataxia (including stumbling);
- inability to stand;
- multiple limb paralysis.

A non-WNV-affected area is any county where WNV infection in equine has not been diagnosed in the current calendar year, or any location more than 10 miles from a positive equine case of WNV infection. Illness in an equine in a non-WNV-affected area should be considered a suspect case if at least one of the following signs is present:

- apprehension;
- depression;
- listlessness;

plus any two of these signs:

- head shaking;
- flaccid paralysis of the lower lip;
- ataxia (including stumbling);
- weakness of hind limbs;
- inability to stand;
- limb paralysis;
- paresis;
- acute death.

A suspect equine case in a non-WNV-affected area should be investigated as a foreign animal disease (FAD). The entire United States is currently considered a non-WNV-affected area.

FAD investigations should be completed in accordance with VS Memorandum 580.4. Specimens should be submitted to the National Veterinary Services Laboratories (NVSL) with an FAD investigation number in order to facilitate tracking and timely reporting of diagnostic results. Airway bill numbers for shipments to NVSL in Ames, Iowa, should be provided to VS Emergency Programs at (301) 734-8073.

Report FAD investigations to the Area Office, Regional Office, and Emergency Programs; these FAD investigations generally will be considered Priority 2. If there are questions or concerns, please contact Emergency Programs.

Sample Submission

Samples for submission to NVSL should be shipped by Federal Express to:

Dr. Eileen Ostlund
NVSL
1800 Dayton Road
Ames, IA 50010

Contact NVSL (phone: 515-663-7551, fax: 515-663-7348) to provide an airway bill number, the number of samples, and relevant epidemiological information.

Antemortem Sample Collection

Collect one serum sample (in a 10 ml red-top tube) and one whole blood sample (in a 10 ml EDTA purple-top tube). Send the serum and whole blood to NVSL.

Postmortem Sample Collection

Use appropriate protective gear when collecting and processing postmortem samples (see "Recommendations for Safe Practices for Conducting Necropsies of Suspected WNV Cases" below).

If a suspect equine is to be euthanatized, collect at least one serum sample (in a 10 ml red-top tube) and one whole blood sample (in a 10 ml EDTA purple-top tube) prior to euthanasia. Send the serum and whole blood to NVSL.

When a postmortem on a suspect equine is performed, the following samples should be collected and sent to NVSL or the State public health laboratory, as indicated:
- Fresh brain tissue (for rabies testing) -- send to State public health laboratory.
- Fresh and fixed brain tissue -- send to NVSL.
- Fresh and fixed spinal cord segments (cervical, thoracic, and lumbar) -- send to NVSL.
- Cervical and lumbo-sacral cerebrospinal fluid (CSF) -- send to NVSL.

Samples collected from the postmortem of a suspect equine and submitted to NVSL for WNV testing will be processed only after the animal has tested negative for rabies according to established protocols in a given State. The foreign animal disease diagnostician should notify NVSL of the rabies test results as soon as they are available.

<u>Recommendations for Safe Practices for Conducting Necropsies of Suspected WNV Cases</u>

WNV is a flavivirus transmitted in nature by mosquitoes. Infection of otherwise healthy people causes a mild febrile illness or no symptoms at all. Mortality has been reported in the elderly; immunocompromised individuals also are at a higher risk.

Although no evidence exists of aerosol transmission, precautions should be taken in laboratory and field settings; the main concern is viral contact with open wounds and mucous membranes. Little is known of the infectious dose for humans or the magnitude and duration of the viremia in different animal species.

Recommendations for Field Necropsy of WNV Suspect Animals:

1. Keep the use of needles and sharp instruments to a minimum.
2. Do NOT use mechanical saws to obtain spinal cord samples. For proper procedures, see "Collection of Spinal Cord Segments" below.
3. Procedures that create an aerosol should be done in a way to minimize the dispersal of the aerosol particles.
4. Wear Tyvek disposable coveralls or, at a minimum, a solid-front, water-resistant, long-sleeve gown.
5. Wear three pairs of gloves. The innermost pair should be latex or other disposable gloves. Substantial waterproof gloves (e.g., Playtex kitchen gloves) should be worn over the innermost pair. The gloves should be long enough for the gown sleeves to be tucked inside the gloves; duct tape may be useful for keeping sleeves inside gloves. The outermost pair of gloves should be metal or Kevlar, e.g., a Whizard Hand Guard (steel/Kevlar) glove from Koch (1-800-456-5624) or a locally purchased filleting glove. THIS OUTER PAIR OF GLOVES MUST BE WORN throughout the necropsy procedure.
6. Wear a face shield or goggles to protect mucous membranes, and wear a disposable "half mask" HEPA respirator (3M 8293) to avoid aerosol infection.

<u>Collection of Equine Brain Tissue</u>

Diagrams showing the procedure for collecting equine brain tissue are reproduced from *Equine Medicine and Surgery*, 3rd ed., 1982, edited by Mansmann, McAllister, and Pratt (see the last page of these guidelines). Always use appropriate protective gear when collecting and processing samples.

<u>Collection of Spinal Cord Segments</u>

Collect spinal cord in 4-centimeter-long segments from cervical, thoracic, and lumbar sites.

Procedures for Obtaining Cervical Spinal Cord Segments:

1. At the vertebral column where the head has been disarticulated, remove the soft tissue from 4 or 5 cervical vertebrae.
2. Depending on the circumstances, it may be advantageous to disarticulate the cervical vertebral column from the rest of the carcass, allowing the specimen to be placed on an elevated surface for further dissection. Assistance may be needed to hold the specimen on an elevated surface for further dissection. Assistance in holding the

specimen steady, in the form of either a person or a vise, will facilitate the remaining steps.

3. Using a manual bone saw, make transverse cuts through the midportion of each of the first four vertebral bodies. This will produce four isolated segments of cervical vertebral column, each containing an intervertebral joint at its center.
4. Observe the isolated vertebral segments from the cut ends, noting the spinal cord held in place by the spinal nerves, which exit the vertebral canal through the intervertebral foramina. Grasp the dura mater with toothed thumb forceps, apply gentle traction, and snip the spinal nerves with long thin scissors (e.g., Metzenbaums). Perform this procedure at each end of the vertebral segment.
5. For sample submission: divide each cervical spinal cord segment in half; fix one half in formalin and maintain the other half as a fresh sample. Ship the fresh and fixed segments to NVSL.

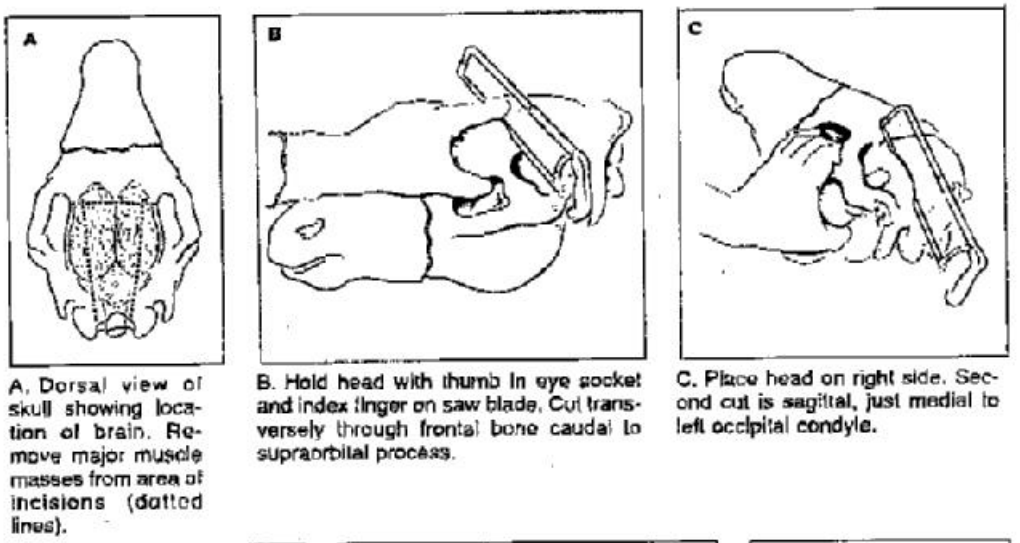
Procedures for Obtaining Thoracic and Lumbar Spinal Cord Segments:

1. Excise and remove the last two ribs.
2. Remove the soft tissue around the thoracic vertebrae that have had the ribs removed. Also remove the soft tissue from around the adjacent lumbar vertebrae.
3. Basically, repeat the steps used for collecting the cervical spinal cord segments by making transverse cuts through the thoracic vertebrae and continuing down through the exposed lumbar vertebrae.
4. Remove the spinal cord segments from the vertebral segments as described for the cervical cord segments.
5. For sample submission: divide each thoracic and lumbar spinal cord segment in half; fix one half in formalin and maintain the other half as a fresh sample. Ship the fresh and fixed segments to NVSL.

<u>Collection of CSF</u>

A good site to collect CSF is at the atlanto-occipital junction just as one cuts through the ligaments prior to decapitation. Up to 15 ml of CSF can be collected from this site. Collect as much fluid as possible. CSF may also be collected from a sacral tap on postmortem. Identify the CSF as to site of collection and submit to NVSL.

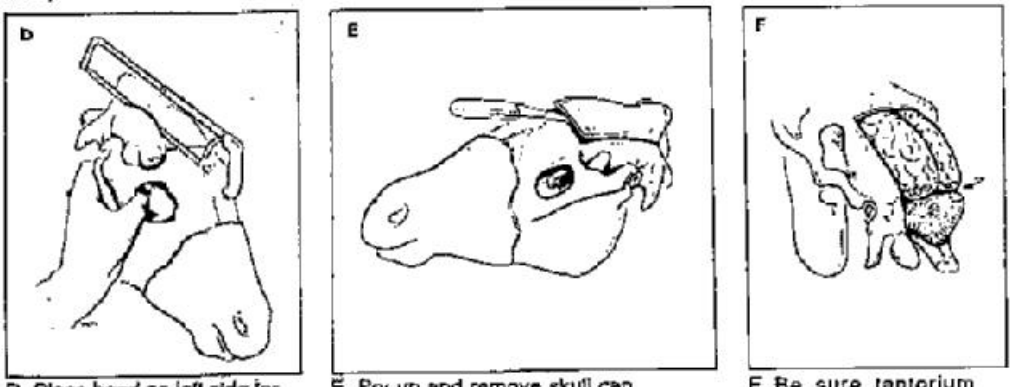
Procedure for Collecting Equine Brain Tissue



A. Dorsal view of skull showing location of brain. Remove major muscle masses from area of incisions (dotted lines).

B. Hold head with thumb in eye socket and index finger on saw blade. Cut transversely through frontal bone caudal to supraorbital process.

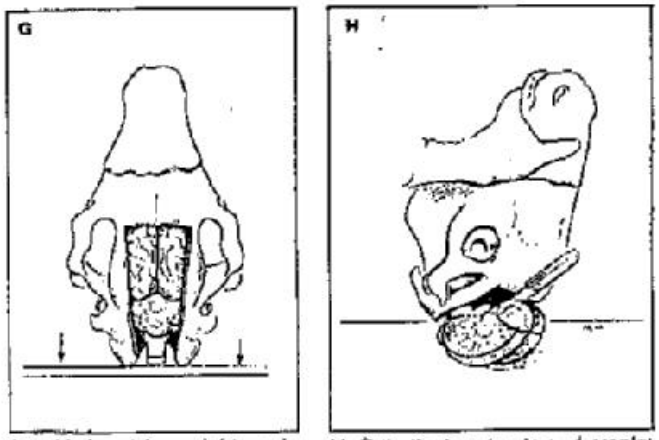
C. Place head on right side. Second cut is sagittal, just medial to left occipital condyle.



D. Place head on left side for right sagittal cut. Place nose toward you, thumb in eye socket and fingers around mandible.

E. Pry up and remove skull cap.

F. Be sure tentorium cerebelli (arrow) and other limiting dura are removed.



G. With head in upright position, tap it lightly on table to loosen brain.

H. Cut olfactory tracts and cranial nerves as brain is removed. Tilt head so that brain rests on table. Section, label and place in formalin.

Fig 8a. Necropsy technic for removal of the brain. [Adapted from Mod Vet Pract 60 (1979) 109]